Discovery of a host fish species for glochidia of *Westralunio carteri* Iredale, 1934 (Bivalvia: Unionoidea: Hyriidae)

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Abstract

Freshwater fishes are the usual hosts of glochidia (the parasitic larval stage) of freshwater mussels (Bivalvia: Unionoidea). *Westralunio carteri* Iredale 1934 (Carter's mussel), the only unionoid species found in the Sout West Coast Drainage Division of Australia, is endemic to the region and is currently listed as Vulnerable on the IUCN Red List, yet nothing is known of its host species. Small, white, bladder-like cysts were observed macroscopically on *Tandanus bostocki* Whitley, 1944 (freshwater cobbler) captured from the Blackwood River, Western Australia. Light microscopy of sectioned cysts revealed that they contained glochidia and these were of similar size and shape to glochidia obtained from gravid females of *W. carteri*. Glochidia were found on 40.7% of 461 *T. bostocki* examined, with a mean intensity of 10.6 cysts per infested fish. Prevalence of infestation was greater on juvenile (based on development morphology or urogenital papilla) than on adult fish. The findings represent an important step in developing conservation measures for *W. carteri* in this region.

Keywords: Freshwater mussel, freshwater cobbler, *Tandanus bostocki*

Introduction

Freshwater mussels, of the order Unionoida, are an ancient group of bivalves found in freshwaters on all continents apart from Antarctica (Bauer & Wächtler 2001). Mussels play important roles in the functioning of freshwater ecosystems, through their biological activities, such as filter feeding, nutrient cycling and biodeposition, and by providing structural habitat and microrefugia to other benthic organisms (Strayer *et al.* 1999; Spooner & Vaughn 2008). Globally, freshwater mussels are highly imperilled, with biotic surveys in many countries demonstrating a general decline in both species richness and overall abundance (Williams *et al.* 1993; Vaughn & Taylor 1999; Strayer *et al.* 2004; Lydeard *et al.* 2008).

Unionoids are dioecious and reproduce sexually; males release sperm into the water column, which females suck in through their inhalant siphons and fertilise eggs that have migrated from the ovaries into specialised pouches in the gills known as marsupia, where the embryos develop into larval glochidia (Bauer & Wächtler 2001; Strayer 2008). Glochidia are released from marsupia, in response to disturbance or other stimuli, and if they contact a suitable host, generally a fish, may attach to the body surfaces, fins, mouth or gills (Bauer & Wächtler 2001; Strayer 2008). Following attachment, glochidia are encased in host epithelial tissue and within the epithelial cyst they undergo metamorphosis to emerge as juvenile mussels (Bauer & Wächtler 2001; Strayer 2008). To facilitate the attachment to fish, some glochidia have specialised structures (known as larval teeth) on the ventral margins of their shells. Teeth vary in morphology, but are generally hooked (Bauer & Wächtler 2001) and can often be used to identify glochidia taxonomically (Jones *et al.* 1986; Jupiter & Byrne 1997).

Eighteen species of unionoid mussels are known from Australia, all from the family Hyriidae (Walker *et al.* 2001; Graf & Cummings 2010). *Westralunio carteri* Iredale, 1934, the sole member of the genus *Westralunio* in Australia, is endemic to the South West Coast Drainage Division, where it is the only freshwater mussel found in the region (Walker *et al.* 2001; Graf & Cummings 2010). The species is currently listed as Vulnerable on the IUCN Red List of Threatened Species (IUCN 1999) and as a Priority 4 fauna (rare or near threatened or in need of monitoring) by the Western Australian Department of Environment and Conservation, under the Wildlife Conservation Act, 1950 (DEC 2010). Detailed understanding of the conservation status of *W. carteri*, and the development of conservation plans, are hampered by an almost complete lack of published information on the life history of the species, including longevity, reproductive cycle, habitat requirements and importantly, host fishes. Furthermore, of four other species of Hyriidae found in north-western and north-eastern WA, only one host fish species has been identified for glochidia of *Velesunio angasi* (Sowerby, 1867) (Klunzinger *et al.* 2010). Knowledge of host fish species, in particular, may be a crucial component of conservation planning for freshwater mussels because fishes are an obligatory part of the mussel life cycle (Haag & Warren 1998; Martel & Lauzon-Guay 2005).

Here we report, for the first time, the discovery of a host fish species, freshwater cobbler *Tandanus bostocki* Whitley, 1944 for the glochidia of *W. carteri*.

Methods

Freshwater cobbler were captured using two-winged fyke nets in four main channel sites and two tributaries in the Blackwood River (between 34.0421°S, 115.6025°E and 34.1081°S, 115.4505°E), in November 2008 as part of the study by Beatty *et al.* (2010). All fish were sexed, where possible, and measured for total length (TL), to the nearest 1 mm. Whitish, bladder-like cysts on the surface of the fish were provisionally identified as containing glochidia, and prevalence (percentage of fish infested) and intensity (number of cysts per infested fish) recorded from field examinations. Ninety five percent confidence intervals were calculated for prevalences, assuming a binomial distribution, and intensities, from 2000 bootstrap replications, using the software Quantitative Parasitology 3.0 according to methods described by Rozsa *et al.* (2000). The effect of maturity status and sex on prevalence was tested using Fisher exact tests and the effect of TL on prevalence by comparing the TL of infested and uninfested fish using analysis of variance (ANOVA). The effect of maturity status and sex (based on developmental morphology of urogenital papilla (Morrison 1988)) on intensity was tested by comparing the intensity of infestation between juvenile and adult, or male and female fish, using ANOVA. The effect of TL on intensity was tested by regression analysis.

A sub-sample of fish (n = 3) was killed in an ice slurry bath and transported to the laboratory. Cysts were examined under a dissecting microscope to determine whether they contained glochidia. Several cysts were preserved in 10% formalin in preparation for histology and scanning electron microscopy (SEM). For histology, dissected cysts were dehydrated in graded ethanols, embedded in paraffin, serially sectioned (6 μm thick) and stained with haematoxylin and eosin. For SEM, dissected cysts were dehydrated in graded ethanols, placed on a glass cover slip attached to a specimen stub, critical point dried, sputter-coated with gold, and examined and photographed in a Philips XL 20 SEM. For comparison, adult *W. carteri* (n = 2) were hand collected from the Canning River (32.1129°S, 116.0170°E), near Perth, killed in 0.01% benzocaine solution and dissected. Glochidia were transferred from gill marsupia with a probe and either examined under a compound microscope or using methods for SEM as described above.

Results and Discussion

Cysts were found on the fins, body surface and gills of *T. bostocki* (Fig. 1). Histological examination confirmed the presence of glochidia in fish cysts (Fig. 2). These were of similar size and shape to the unattached glochidia removed from specimens of *W. carteri* (Figs 3 and 4), although using morphology to identify encysted glochidia to species is difficult because the larval teeth are usually not visible (Jupiter & Byrne 1997). In unattached glochidia, these teeth appeared as two separate interlocking hooks on the ventral edges of glochidial valves (Fig. 3); somewhat similar to those described for another Australian unionoid, *Hyridella depressa* (Jupiter & Byrne 1997). Glochidial teeth function as a mechanism for attachment to host fish, and Pekkarinen & Englund (1995) found that glochidia with well-developed teeth are often attached to fins and skin rather than gills of fish. We assume that the encysted glochidia found on *T. bostocki* are *W. carteri* because this is the only unionoid species that has been described from the south-west of Western Australia (Walker *et al.* 2001; Graf & Cummings 2010).

Of 461 *T. bostocki* examined (107 males, 268 females and 86 unsexed, presumably juveniles) from the Blackwood River in November 2008, glochidial cysts were found on 40.7% (95% CI = 36.3 – 45.2). Of the 107 male *T. bostocki* examined, glochidia were found on 34.6% (95% CI = 32.2 – 44.2). Of the 268 female *T. bostocki* examined, glochidia were found on 38.1% (95% CI = 25.6 – 44.4). There was no significant difference in prevalence between male and female fish (Fisher exact test, d. f. = 1, P = 0.56). Of the 86 juvenile *T. bostocki* examined,

Figure 1. (a) Glochidia cysts, appearing as white, raised areas on the caudal fin of a female *Tandanus bostocki*. (b) Individual cyst on the left dorsal side of the dorso-caudal fin of *T. bostocki*.

Figure 2. Section of a cyst (Cs), showing glochidium (G) with shell periostracum (Po), covered by fish epithelium (E), from the posterior end of the dorso-caudal fin of *Tandanus bostocki*.

Figure 3. Light microscope image of glochidium of *Westralunio carteri*; anterior-ventral view, valves open, larval teeth shown.

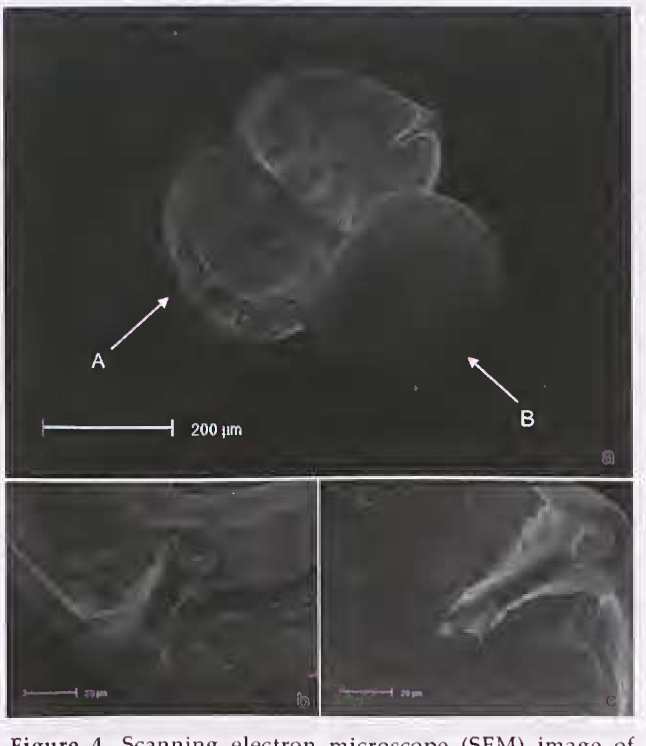

Figure 4. Scanning electron microscope (SEM) image of glochidia of *Westralunio carteri*. (a) Ventral view, valves open (A) and right valve view, valves closed (B). (b) Larval tooth of left valve, magnified image of (A). (c) Larval tooth of right valve, magnified image of (A).

glochidia were found on 57.0% (95% CI = 45.9–67.3), which was significantly greater than on adult fish (Fisher exact test, d. f. = 1, P = 0.001). Considering adult and juvenile fish separately, there were no significant differences in TL between infested and uninfested fish (for adults, F = 0.02, d. f. = 1, 374, P = 0.89; for juvenile, F = 3.29, d. f. = 1, 85, P = 0.07). Of infested fish, the intensity of infestation varied from 1–120, with a mean of 10.6 (95% CI = 9.0–12.5). There were no significant differences in intensity between male and female fish (F = 0.01, d. f. = 1, 374, P = 0.97) or between adults and juveniles (F = 2.41, d. f. = 1, 460, P = 0.12), nor were there any significant relationships between TL and intensity for adult or for juvenile fish (for adults, r^2 = 0.001, F = 0.37, d. f. = 1, 374, P = 0.54; for juveniles, r^2 = 0.01, F = 0.69, d. f. = 1, 85, P = 0.41).

Studies on other unionoid species have usually found greater prevalences of infestation on juvenile fish than on adult fish, presumably because of the development of immunological resistance in older fish (*e.g.* Bauer 1987; Hastie & Young 2001). The difference in prevalence of *W. carteri* glochidia between adult and juvenile *T. bostocki* suggests that immunological responses could also be important in this host/parasite system, although further research is required to determine the survival rates of glochidia on fish of different age classes. Variation in glochidia prevalence and intensity may also arise from habitat structure (giving rise to variations in the probability of contact between glochidia and potential hosts), fish abundance, seasonality of glochidia release, differences in mussel density and water depth (Strayer 2008).

Globally, most freshwater mussel species that have been examined, and particularly those with hooked glochidia, have been found to be host generalists, with a number of host fish species involved in the life-cycle (Haag & Warren 1998; Wächtler *et al.* 2001; Martel & Lauzon-Guay 2005; Blažek & Gelnar 2006). Although there have only been a few studies of host fish species for Australian unionoids, these have also identified multiple host species (Hiscock 1951; Atkins 1979; Walker 1981; Humphrey 1984; Widarto 1993; DPIPWE 2009; Klunzinger *et al.* 2010). It seems likely, therefore, that fishes other than *T. bostocki* may be infested with glochidia of *W. carteri* in the south-west of Western Australia, although they were not detected in a recent survey of the parasites of native and exotic fishes in the region (Lymbery *et al.* 2010). Furthermore, there are river systems within the region that contain *W. carteri* but do not contain *T. bostocki* (Morgan *et al.* 1998; Klunzinger unpublished data). Understanding the range of host fish species used by *W. carteri* in different systems, and their relative importance in maintaining the life-cycle of the species, is vital for conservation planning, because of the restricted distribution and threatened nature of many of the native fish species of the south-west of Western Australia (Morgan *et al.* 1998; Morgan & Gill 2000; Morgan 2003; Beatty & Morgan 2010).

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